

# Stem-cell therapies for blood diseases

**For decades, transplantation of haematopoietic stem cells — either unmodified, or genetically modified to correct genetic disorders — has been used to treat disorders of the blood and immune systems. The present challenge is to reduce the risk of such transplants and increase the number of patients who can safely access this treatment. In developing countries, such 'one-shot' treatments are highly desirable because chronic treatments are difficult to sustain. To make these therapies more accessible and effective it will be important to improve clinical protocols and gene-delivery vectors, and to gain a deeper understanding of stem cells.**

Since the 1950s and 1960s, bone-marrow transplants, in the form of intravenous infusions of whole marrow, have been used to treat patients with acute leukaemia, bone-marrow aplasia and congenital immune deficiencies. These clinical treatments led to basic studies to identify, isolate and characterize the rare stem cells (haematopoietic stem cells, or HS cells) that populate the cellular component of the marrow and peripheral blood, and to understand their therapeutic potential. This work has been seminal in the field of stem-cell research, and has provided reagents and models for other tissue-specific and organ-specific stem cells. It was recognized by the 1990 Nobel Prize awarded to E. Donnall Thomas. Despite these successes, the wider therapeutic application of HS-cell transplantation to blood disease is limited by the lack of suitable donors, the high rate of mortality associated with the procedure and the recurrence of the underlying diseases. Here, we discuss the limitations of current HS-cell-transplant therapies, and review efforts to improve their therapeutic potential and availability to treat a wider spectrum of patients suffering from diseases of the blood.

## **HSCs in health and disease**

Bone marrow was, for many years, transplanted as an unfractionated tissue, until scientists discovered which cellular components were responsible for the engraftment of the donor haematopoietic and immune systems in marrow-ablated patients. These cells, the HS cells, have turned out to be among the most active in the human body. As the cellular and subcellular components of the blood (such as white cells, red cells and platelets) have life spans of only days or months, HS cells must continually produce new ones. Under normal physiological conditions, quiescent blood stem cells are interspersed with differentiated cells within the marrow of flat and short bones. However, under conditions of stress, such as massive bleeds or acute bacterial infections, HS cells respond rapidly by accelerating their proliferation, differentiation and migration from the marrow to circulate throughout the body. HS cells are so potent that, even after an injury has wiped out most of the peripheral blood and marrow cells, it takes only a small number to fully restore a functional haematopoietic system. In mouse models, less than one hundred highly purified HS cells can fully reconstitute the blood system of recipient animals after the bone marrow has been completely ablated by radiation. In experiments in mice, serial transplantation of HS cells — in which they are isolated from the marrow of the recipient and used to reconstitute a second marrow-ablated recipient—can be repeated several times before stress on the original HS cells leads to incomplete reconstitution. The powerful growth and expansion properties of HS cells make them attractive candidates for a range of clinical applications. There is also great progress in methods for treating patients with growth hormones in order to expand HS cells and the HS-cell population, and mobilize them from the bone marrow into peripheral

blood, from which they can be readily isolated in relatively high numbers. Therapies for HS-cell mobilization have the advantages of avoiding the complex and invasive procedure of bone-marrow explant under general anaesthesia, while providing higher yields of purified HS cells. Such improvements have even made it possible to perform transplants from partially incompatible donors, and have contributed to HS-cell transplantation becoming the treatment of choice for a number of genetic and acquired disorders.

### **Complexities of HS-cell transplantation**

Successful HS-cell transplantation relies on a complex interplay of factors, including an intact bone-marrow niche, stem-cell competition, reaction of donor immune cells to diseased or malignant cells of the recipient, and efficacy of treatments designed to eliminate diseased cells prior to transplant. Successful engraftment of HS cells has been shown to require an intact marrow environment (or niche), which provides the signals for homing, seeding and expansion of the transplanted stem cells. Studies of bone-marrow transplants used to treat leukaemia have shown that immune recognition and elimination of leukaemic cells by the donor immune system have central roles in patient outcome — a condition called graft-versus-leukaemia. Thus, leukaemic recurrence in bone-marrow transplantation can depend on the degree of identity of genes that regulate tissue compatibility between donor and recipient, with the highest rate of recurrence in genetically identical twins. The most confounding limitation of bone-marrow and HS-cell transplantation is the availability of appropriate donors. The ideal donors are healthy histocompatible family members, who are available for only about one-third of patients. Despite efforts to create international registries of unrelated donors and HS-cell repositories from umbilical cord blood, a substantial proportion (up to 50%) of patients have no access to potentially life-saving transplants owing to a lack of suitable donors. In recent years, technologies for purifying and transplanting HS cells have made it possible to perform transplants from half-matched family donors, who are available for almost all patients (the parents and children of any individual are genetically half-matched). Unfortunately, the hopes that HS-cell transplantation could provide a solution have been frustrated by unacceptably high rates of transplant-related mortality in recipients of such half-matched HS-cell transplants. Whereas highly purified HS cells from half-matched donors can reconstitute a functional bone marrow and nearly all cellular components of the blood in a marrow-ablated patient, they fail to provide the lymphocytes necessary for rapid and complete immune reconstitution. Thus, the patient remains unprotected against potentially lethal viruses and other infectious agents, and has a high risk of dying from infection. Attempts to overcome this problem by combining stem-cell transplantation with infusions of unmanipulated lymphocytes from the donor have been thwarted by the tendency of donor immune cells to react against the patient's organs and tissues. Such reactions are often lethal because of the high degree of mismatch between the donor and patient. Strategies for immune protection of the recipient by the use of defined subsets of lymphocytes are being developed and tested in mouse models. Alternatively, genetic manipulation of donor lymphocytes to insert a suicide-gene system has been shown to control this complication in patients. It is hoped that such strategies will eventually allow HS-cell transplants for many more patients with malignant diseases of the haematopoietic system and lethal genetic disorders.

### **HS-cell transplantation for the developing world**

Developing countries of the Far and Middle East and Africa have large numbers (believed to be tens of thousands) of patients affected by lethal red-cell disorders. One such disease is thalassaemia major, which is a genetic disorder that affects

haemoglobin. In Westernized countries, it is treated by packed red-cell transfusions from selected blood donors, which are administered periodically throughout the lifetime of the patient; these are combined with chronic drug therapy for preventing overload of the iron present in the red-cell transfusions. In most underdeveloped countries, however, there are no national blood-donor programs, and thalassaemic patients die in childhood. Thus, purified HS cells from family donors could potentially provide an affordable therapy where transfusion programmes are unavailable. In this situation, HS-cell transplantation could not only offer a technologically advanced treatment with the best chance of curing the disease, but could also be used to treat relatively large numbers of patients. It is true that HS-cell transplantation is still an expensive procedure that carries a variable, but significant, risk of mortality. In the future, however, it is hoped that by simplifying HS-cell isolation and implementing less-toxic preparative regimens for patients, the expense and mortality of transplants will be reduced. Currently, most HS-cell transplantation for Middle Eastern thalassaemia patients is performed in Western countries with dedicated programs, such as that of Fondazione IME, an international health organization that has the principal responsibility of carrying forward the International Thalassaemia Project. If more international collaborations such as this could be established, the lives of thousands of young patients could be saved by such one-shot therapies, which might provide a permanent cure rather than life-long, expensive and poorly tolerated treatments.

### **HS-cell gene therapy**

Gene therapy offers an alternative to stem-cell transplantation from healthy donors for the treatment of thalassaemia and certain other genetic diseases of the blood. In gene-therapy approaches, a patient's own HS cells are isolated from bone marrow or blood, genetically engineered to express a normal copy of the gene responsible for the disease and then transplanted back into the patient. Since the early 1990s, this type of gene therapy has been successfully used to treat severe combined immunodeficiencies (SCIDs), using viral vectors to carry a healthy gene into the genome of the patient's HS cells. Patients affected by the X-linked variant, the adenosine deaminase (ADA)-deficient variant and chronic granulomatous disease seem to be permanently cured of their defects using this strategy. Unfortunately, despite these clinical successes, HS-cell gene therapy for genetic diseases has not progressed at the expected pace. One reason is the difficulty involved in appropriately regulating expression of the transferred genes, which is a particular problem for the globin genes. Another reason is related to the presentation of a severe adverse event in a clinical study of X-linked SCID. In the clinical study, three patients, after obtaining optimal clinical correction of the disease, showed a progressive lymphoid proliferation that clinically resembled an acute leukaemia. This adverse event could be directly linked to a specific clonal insertion of the viral vector used for gene transfer into the stem-cell host genome near a known oncogene. Despite the fact that this event seems to have several unique features associated with this specific trial (a similar study performed by a different group did not show any adverse event), all stem-cell gene-therapy trials were subjected to intense scrutiny and were put on hold for varying periods of time in most countries. We expect that HS-cell gene therapy will progress at a faster pace now that safer and more efficient vectors are being produced and are entering clinical trials. In addition to congenital immunodeficiencies, a number of other genetic diseases of the blood might become candidates for such treatments. In particular, neurodegenerative disorders caused by the absence of specific enzymes, such as lysosomal enzymes, are good candidates for HS-cell gene therapy, as replacement enzymes can potentially be produced by cells generated from genetically engineered HS cells.

### **HS-cell plasticity**

*In addition to the classical application of HS cells for treating blood diseases, there has been great interest in the idea of using them to treat diseases of other organs and tissues. Engraftment, and in some cases tissue repair, has been reported in a range of organs after systemic or local injection of HS cells or bone marrow. Initially it was thought that the HS cells responded to local stimuli by differentiating into mature cells of the recipient organ. Later, it was found that cell fusion was a major mechanism for stem-cell contribution to the regeneration of the treated organ. Although the jury is still out on the actual role of trans-differentiation in stem-cell-mediated tissue repair, cell fusion represents a more widely accepted general phenomenon, particularly for tissues possessing highly fusogenic cells, such as muscle and liver. It is interesting that, in some cases, therapeutic benefit has been observed in the absence of significant levels of stem-cell engraftment. This apparent paradox can be explained by the ability of stem cells to produce stimulatory and regulatory cytokines that stimulate tissue growth and repair.*

### **In Conclusion**

It is remarkable that, through bone-marrow and HS-cell transplants, stem-cell therapies have brought about permanent cures for some (tens of thousands) patients suffering from blood disorders. Unfortunately, for other patients, there remains a 10–50% rate of transplant related mortality and only a small chance of finding a suitable donor. Thus, transplant medicine seems far from offering an optimum cure for the majority of patients with blood disorders. There are efforts underway to develop therapies using alternative sources of stem cells, such as embryonic stem cells. However, because HS cells are relatively abundant and accessible, alternative sources might be less crucial for treating common blood disorders than for diseases of other organs and tissues. Thus, the most immediate improvements will probably be achieved by developing less-toxic drug regimens for use in preparing the patient to accept the transplant and strategies for prompt immune reconstitution following transplant. Genetic engineering has already been successfully implemented in HS-cell therapy. However, more effective vectors and a better understanding of the consequences of vector integration are needed to expand the number of diseases that can be treated by this technology. Whereas genetic diseases are the most obvious candidates for HS-cell therapies, they might also be effective in treating acquired diseases, such as cancer and AIDS, particularly for patients living in countries where the costs of life-long chronic treatments are unsustainable.

For more information, please see the other reference materials downloadable from our website, or contact us for more in-depth discussions on the use of stem cells in different therapeutic areas.